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Conjugation in *Tetrahymena*: Its Relation to Concanavalin A Receptor Distribution on the Cell Surface

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ABSTRACT—Cell surface events during conjugation of *Tetrahymena thermophila* were studied by electron microscopic examination of ferritin conjugated concanavalin A (F-Con A). Small amounts of ferritin particles (F-particles) were bound to the surface of cells in the nutritional and starved states, but there were no large clusters of F-particles. In regions where F-particles were scantly, the ectoplasmic layer (epiplasm) was directly under the plasma membrane and no alveoli were observed. In contrast, in cells during co-stimulation, large clusters of F-particles were seen on the presumptive junctional area (PJA) formed after the onset of co-stimulation between the complementary mating types, and on the side walls of ectoplasmic ridges of the oral apparatus. In regions of large clusters of F-particles, there was a thick, dense ectoplasmic layer under the plasma membrane and no alveoli were seen. In conjugants, F-particles were seen not only on the smooth PJA but also in zones of gaps in the junctional area between the two conjugants. These findings suggest that the Con A binding glycocalyx is anchored to the ectoplasmic layer, a kind of cytoskeleton, under the plasma membrane.

INTRODUCTION

The protozoan ciliate *Tetrahymena* usually multiplies asexually by binary fission, but it can also reproduce sexually through conjugation. Conjugation can be induced artificially and distinguished into two successive processes: (i) an initiation process induced by removing nutrients from the culture medium, and (ii) a co-stimulation process which begins on mixing the two complementary mating types [1, 2]. Recent studies on the ultrastructure of cells during the conjugation process have shown that complementary mating type cells recognize each other during the co-stimulation period and then interact to form a presumptive junctional area (PJA) on the front side of the anterior region.

This PJA is a special area that has no particular subpellicular organelles. The co-stimulation period is followed by the conjugation period in which the cells pair at the sites of their PJAs. Finally, the PJAs of the conjugated paris partially fuse to form intercellular bridges [3, 4].

On the basis of these findings, the co-stimulation process in this protozoan ciliate can be regarded as the period for formation of a specialized membrane area, which may be important in cell recognition, cell adhesion, and final cell fusion. Glycocalyx on the surface of the plasma membrane is thought to be involved in cellular events such as differentiation, malignant transformation, and cell-cell adhesion [5].

Addition of concanavalin A (Con A) to cultured mammalian cells induces gathering of Con A receptors to form a cap-like structure [6]. Indeed, the conjugation process of *Tetrahymena,* which includes cell transformation, recognition and adhesion, is inhibited by Con A treatment [7, 8].

A previous report from this laboratory described ultrastructural changes in *Tetrahymena* during the process of PJA (SSA) formation [3]. In the present study, ferritin conjugated concanavalin A (F-Con A) was used to examine the distribution of Con A receptors on the cell surface of *Tetrahymena.*

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MATERIALS AND METHODS

The two complementary mating types used were II and IV (strain B) of *Tetrahymena thermophila*, which were kindly provided by Dr. T. Sugai, Ibaraki University, Mito, Japan.

The ciliates were cultured axenically in growth medium (2% proteose peptone, 1% yeast extract, and 0.6% glucose) at 26°C. Late log phase ciliates (10^6 cells/ml) were harvested and washed three times with medium consisting of KCl (0.008%), NaCl (0.2%) and CaCl (0.012%) in redistilled water with low speed centrifugation. Equal volumes of competent cells were mixed to induce subsequent conjugation.

For determination of the distribution of Con A receptors on the cell surface, a suspension of the cells was incubated with 25 μg/ml of F-Con A (E. Y. Laboratories) for 20 min at 26°C. Then the cells were collected by centrifugation and washed twice with 0.2 M phosphate buffer, pH 7.4.

Specific binding of Con A to its receptors was confirmed in a control experiment using α-methyl-mannoside (20 mM).

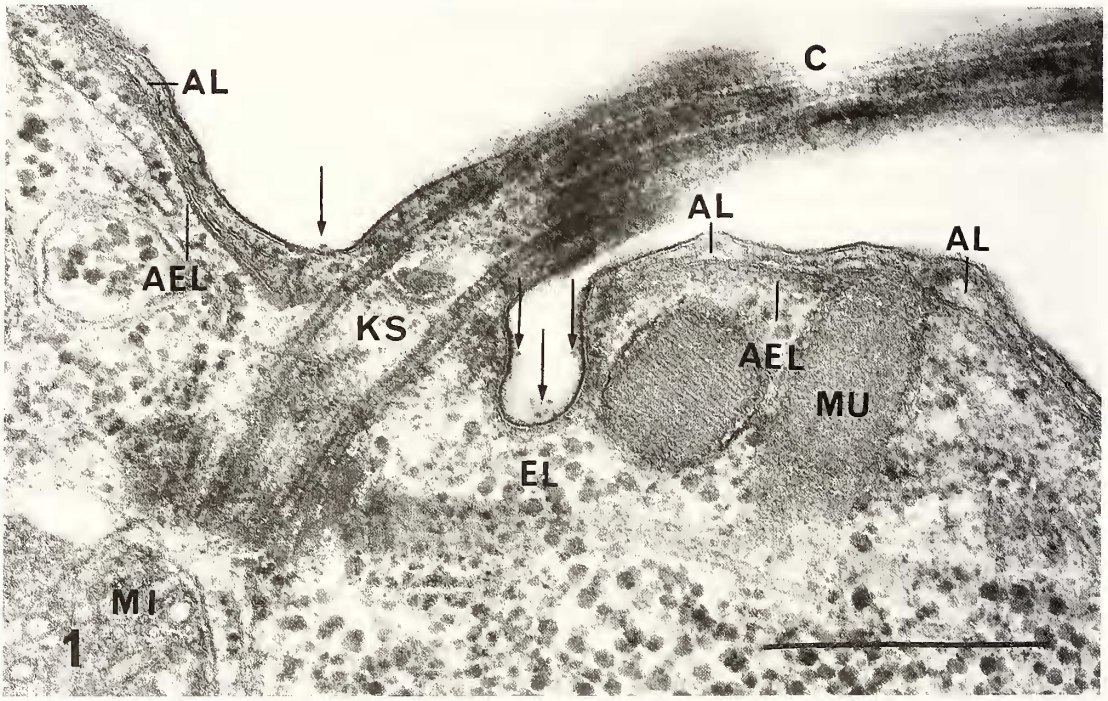
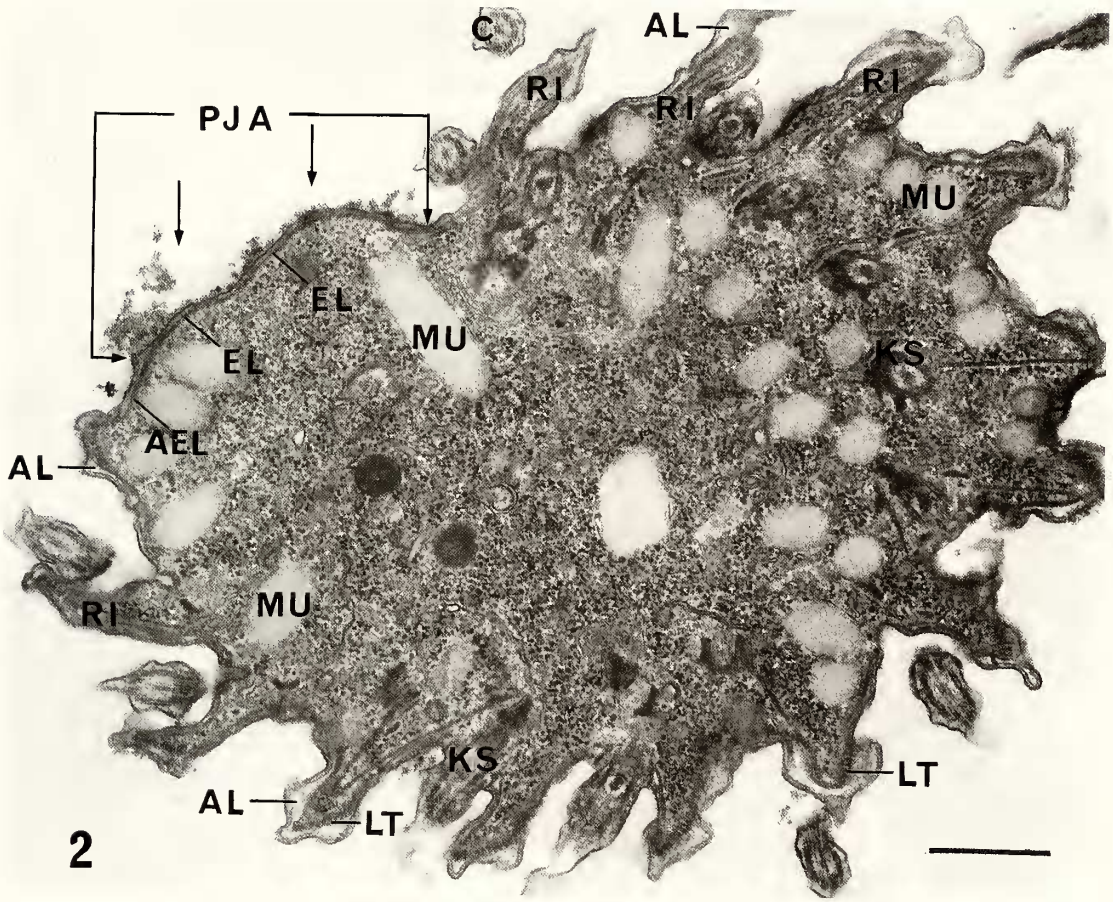


FIG. 1. Section of a starved cell after incubation for 20 min with F-Con A. A few ferritin particles (F-particles) can be seen at the bottom of the ectoplasmic grooves at the ciliary base (arrows). The plasma membrane in this area is directly above a thin ectoplasmic layer (EL). Scale bar: 0.5 μm.

FIGS. 1–7 are transmission electron micrographs of *Tetrahymena thermophila*.
AL: alveoli, AEL: inner alveolus ectoplasmic layer, AZM: adoral zone of membranelle, C: cilium, CI: cisterna, KS: kinetosome, LT: longitudinal tubule, MI: mitochondria, MU: mucocyst, RI: ectoplasmic ridge.

FIG. 2. Cross section through the upper part of the oral apparatus. The cells were mixed for 40 min, then incubated with F-Con A for 20 min. Numerous large clusters of F-particles can be seen on the surface of the plasma membrane of the smooth presumptive junctional area (PJA), but elsewhere there are few particles on the surface. Scale bar: 0.5 μm.

FIG. 3. Section of a closely adjacent part of the same cell as shown in Fig. 2. The EL in the PJA is connected with the inner alveolus ectoplasmic layer (AEL). Clusters of F-particles are found only on the plasma membrane of the PJA that is directly above the EL. Scale bar: 0.5 μm.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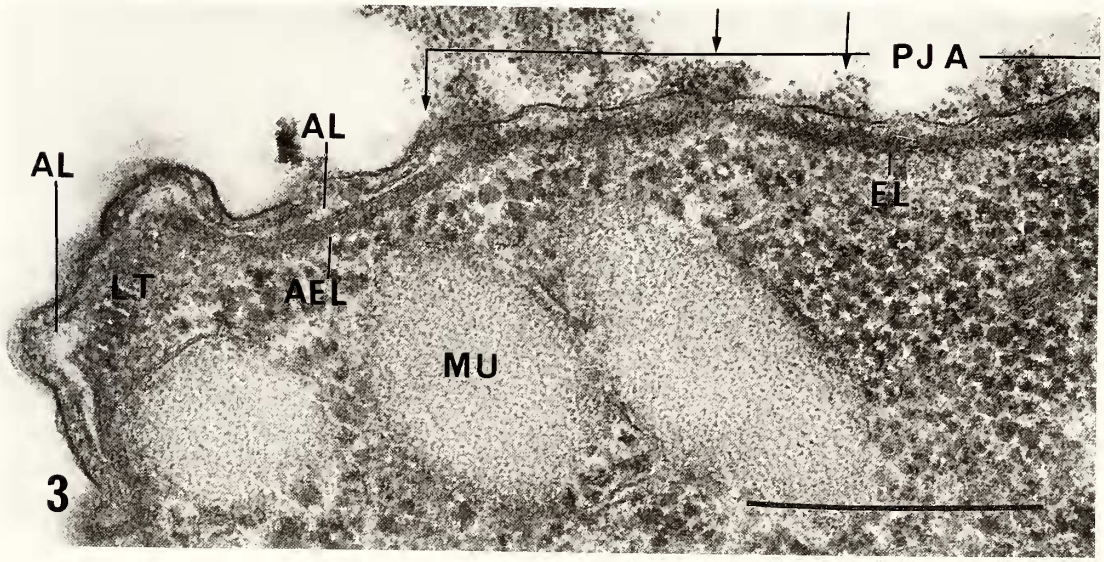
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Samples for electron microscopy were collected by low-speed centrifugation and fixed for 30 min at 0°C in freshly prepared fixative consisting of a mixture of solutions of 0.6% glutaraldehyde, 2% osmium tetroxide, and 1.2% potassium bichromate (2:1:1, v/v) adjusted to pH 7.4 with 0.2 M phosphate buffer. Then they were dehydrated by rapid passage through a graded ethanol series and embedded in Epoxy resin containing Quetol 812 (11 g), DDSA (6 g) and MNA (5.8 g). Ultrathin sections obtained with an LKB ultratome were stained with 1% aqueous uranyl acetate and lead citrate and examined with a JEOL 100-C electron microscope.

OBSERVATIONS AND RESULTS

Tetrahymena cells are pear-shaped, and are covered with numerous ridges stretching along the apse line. Cilia are arranged in a line along the ridges at the bottom. Cross sections, between the tip and upper edge of the oral apparatus of starved cells, have a highly undulating, ellipse-shaped profile. The region below the cytoplasmic membrane is mainly occupied by alveoli, kinetosomes, mucocysts, longitudinal tubules and other subpellicular organelles.

In the starved cell after incubation for 20 min with F-Con A (Fig. 1), a few F-particles are seen located exclusively around the ciliary base (arrows). In the areas where the F-particles are attached, there are no alveoli and an extremely thin ectoplasmic layer (EL) is seen directly below the cytoplasmic membrane. After co-stimulation for 40 min and additional incubation for 20 min with F-Con A, the unpaired cell has the profile of a highly undulating ellipse in cross section between the tip of the cell and the upper edge of the oral

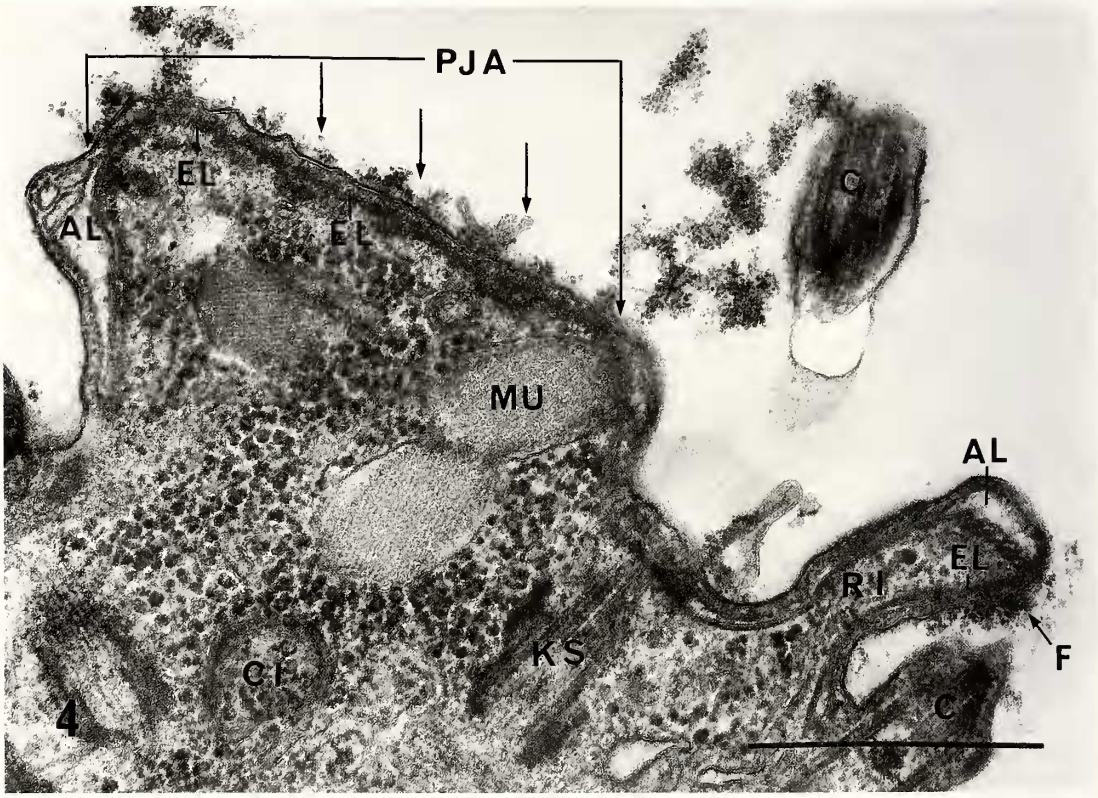

FIG. 4. Cross section through the adoral zone. Cells were mixed for 40 min and then incubated with F-Con A for 20 min. Large clusters of F-particles can be seen on the surface of the plasma membrane of the PJA (arrows) and on the side wall (F) of the ectoplasmic ridge (RI). Scale bar: 0.5 μm.

apparatus (Figs. 2 and 3), like that of cells in the starved state (Fig. 1). But unlike during starvation the PJA appears during co-stimulation. A thick, dense EL appears under the cytoplasmic membrane of the PJA, and F-particles are only found on the surface that is directly above the EL, in areas of alveoli (AL), longitudinal tubules (LT) or cilia (C). The EL is seen as a single dense layer just beneath the cytoplasmic membrane of the PJA, whereas in areas around the PJA, it is displaced deep under the inner alveolus membrane (Figs. 2 and 3, AEL). The ectoplasmic layer is much thicker in cells in the co-stimulation period than in starved cells. Figure 4 shows a cross section through the adoral zone of an unpaired cell. There are numerus F-particles on the PJA (arrows) and on the side wall (F) of the adjacent ridges (RI). The cytoplasmic membrane associated with F-particles is directly above the EL and there are no other subpellicular organelles in the cortical zone.

After co-stimulation for 60 min and incubation for 20 min with F-Con A (Figs. 5 and 6), cross sections through the junction area (JA) of conjugants shows that numerous F-particles are specifically distributed on regions of the PJA, that are not in contact, on the side walls of ridges (arrows) and in gaps between conjugant cells (Fig. 6, F). Figure 7 shows the PJA of a conjugant, without F-Con A incubation. The clear gap zone between the conjugant is filled with fine fibrous structures, possibly glycocalyx.

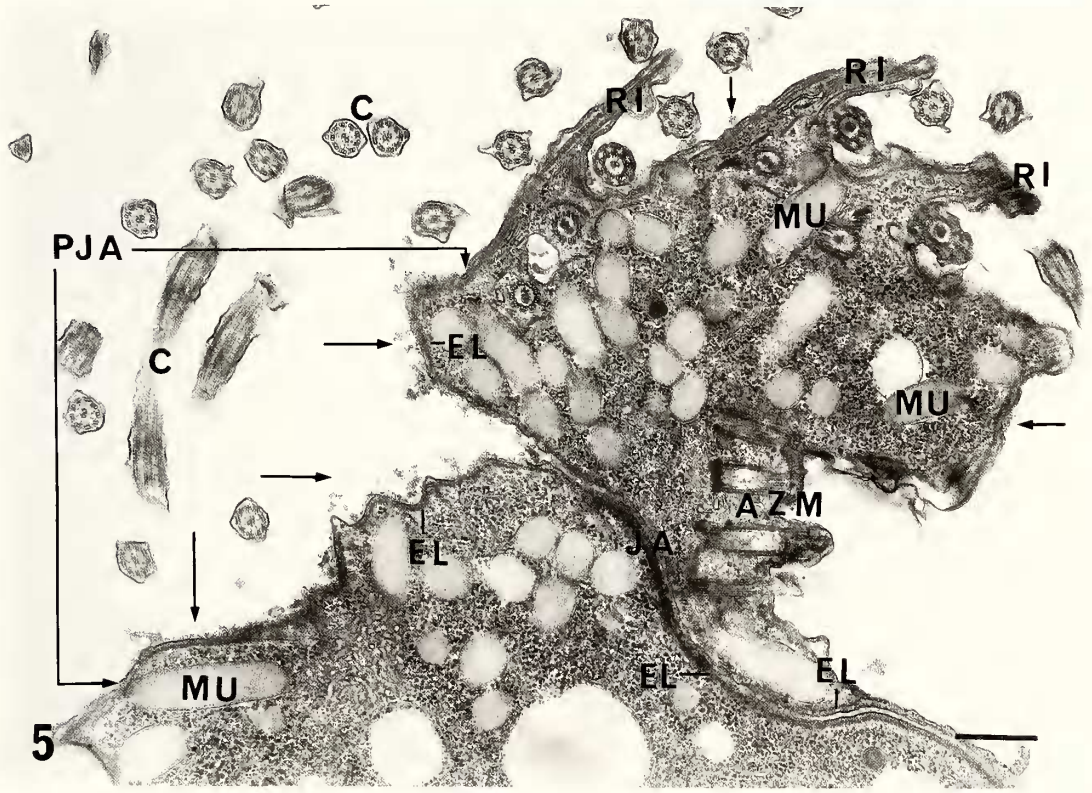

FIG. 5. Cross section through the junction area (JA) of a conjugant. The cells were mixed for 60 min, and then incubated with F-Con A for 20 min. Clusters of F-particles (arrows) can be seen on the surface of the PJA (arrows). Scale bar: 0.5 μm.

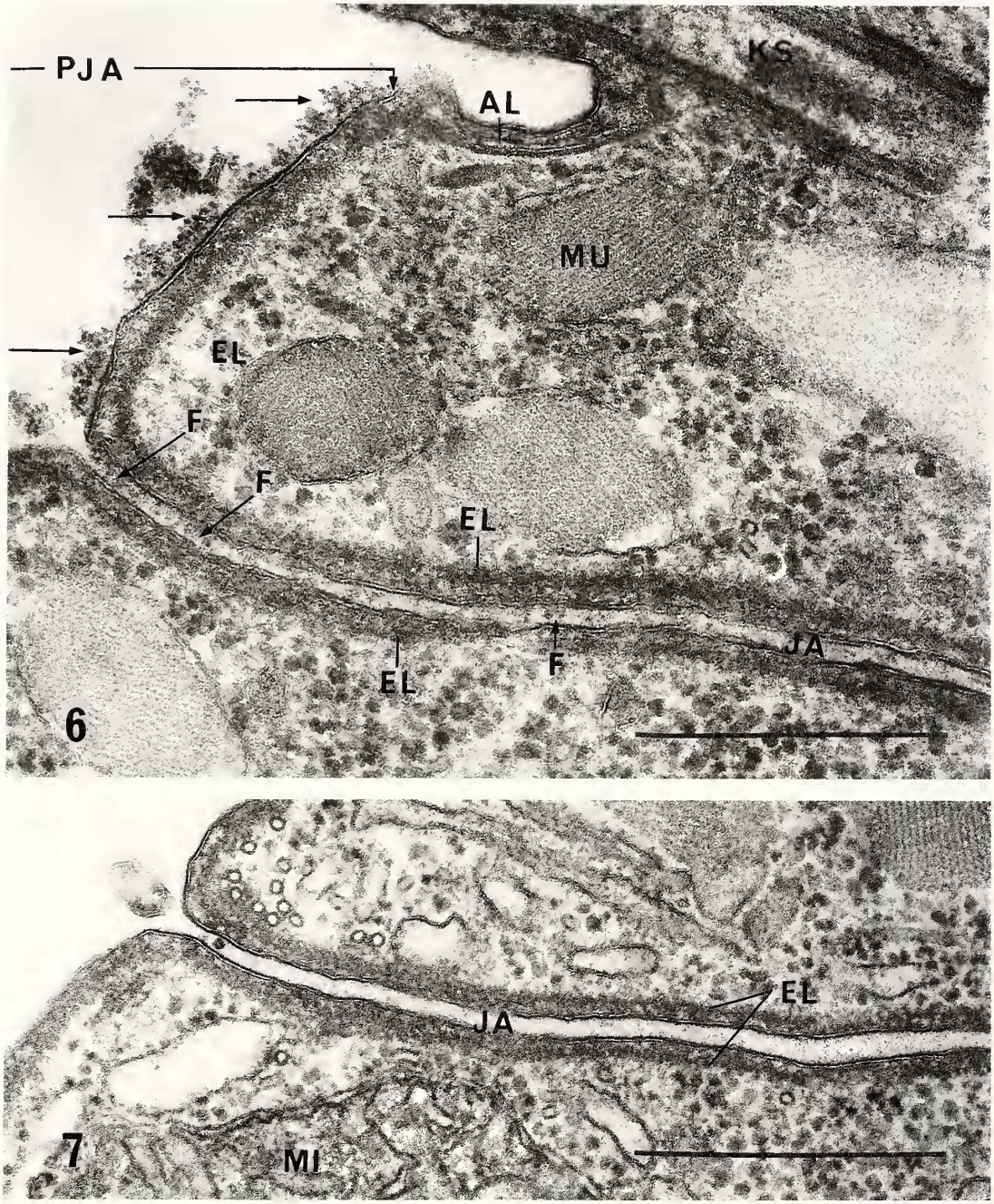

FIG. 6. Section of the junction area (JA) of conjugants. The cells were mixed for 60 min, and then incubated with F-Con A for 20 min. F-particles can be seen singly or in clusters on the surface of the PJA (arrows) and in the gap zone between the conjugants (F). Scale bar: 0.5 μm.

FIG. 7. Section of the junction area of conjugants. The cells were mixed for 60 min. The gap zone between the conjugants (JA) contains many fine fibrous structures. Scale bar: 0.5 μm.

DISCUSSION

The present study on the surface of the cytoplasmic membrane of *Tetrahymena* revealed the distributions of Con A receptors in starved and conjugation-induced cells. The relationship of the distribution of Con A receptors with that of substructures in the cortical zone is noteworthy.

The cortical zone of *Tetrahymena* contains various subpellicular organelles. The cytoplasmic membrane can be classified into the following three membrane areas according to differences in substructures in the cortical zone; (i) a ciliary area, (ii) a cortical area, and (iii) an area directly above the EL. The third type exhibits Con A binding activity specifically. During periods of nutrition and starvation, the third type is found only in restricted areas around ciliary bases and cell membranes that are directly above a thin EL.

There are only a few sparsely distributed F-particles bound to the surface of such areas, so their Con A binding activity may be extremely weak. In studies by fluorescence microscopy with FITC-Con A, no Con A binding activity was detected on the cell surface during starvation before mixing complementary mating types [4, 9]. Since there were so few Con A receptors at the ciliary bases, no FITC could be detected in these regions by fluorescence microscopy.

The most striking ultrastructural changes of the ectoplasm and cortex that occur after mixing starved complementary mating types are thickening of the EL under the inner-membrane of the alveoli and formation of PJAs [3]. Allewell and others [10] proposed that the co-stimulation period should be distinguished into an activation period and maturation period. Morphologically, the former corresponds to the period of thickening of the EL and the latter to the period of PJA formation. The EL under the inner-alveolus membrane, which has thickened and increased in electron density, is morphologically similar to the EL in the PJA, and may be of similar composition to the latter. Surface areas displaying structural similarities to PJA's are also found on the side walls of some cytoplasmic ridges in the cell tip near the adoral zone.

Numerous ferritin particles are attached as large clusters to the outer surface of these special areas as well as to PJAs. After the co-stimulation period, cells can make contact with each other by forming PJAs. When the cells are in partial contact stage of conjugation, however, broad areas of PJAs around the junctional region of the one partner remain free from contact with PJAs of the other cell.

Changes in FITC-Con A binding patterns during the conjugation process were reported by Watanabe *et al.* [11, 12]. The changes in the fluorescence pattern they observed are similar to those in the F-Con A distribution pattern observed in the present study. The ring pattern of FITC-Con A described by Watanabe *et al.* may correspond to the present F-Con A distribution pattern in regions where PJA's of adjacent cells are not in contact.

From previous studies with inhibitors of protein synthesis, a special kind of protein was concluded to be synthesized during the co-stimulation period [13]. This protein was proposed to be a glycoprotein [14]. Watanabe *et al.* [11] found that changes in the Con A binding pattern are stopped or eliminated by cycloheximide. The striking thickening of the EL during the activation period may thus reflect an increase in structural protein in the EL during this period. The close relationship between the distribution of Con A receptors on the cell surface and morphological alterations of the EL under the cytoplasmic membrane strongly suggests that the structural protein(s) is bound to the Con A binding glycocalyx on the cytoplasmic membrane.

Since Con A receptors are rarely found in starved cells, the interaction between cells during the activation period is unlikely to be mediated by Con A receptors; rather, Con A receptors anchored to the EL are likely to play some role in adhesion during the maturation period.

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